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## An Assessment of Stable Hydrogen-Isotope Analysis Methods to Assign Geographic Origin to Migratory Red-Tailed Hawks (*Buteo jamaicensis*)

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AN ASSESSMENT OF STABLE HYDROGEN-ISOTOPE ANALYSIS METHODS TO  
ASSIGN GEOGRAPHIC ORIGIN TO MIGRATORY  
RED-TAILED HAWKS (*BUTEO JAMAICENSIS*)

by Carla Marie Ahlschwede

AN UNDERGRADUATE THESIS

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Majors: Environmental Studies:  
With an emphasis in Natural Resources  
Fisheries and Wildlife:  
With an emphasis in Wildlife Ecology and Management  
And a Water Science Minor

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Dr. Larkin Powell

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## **Abstract**

Stable-hydrogen isotopes are becoming an increasingly popular method of studying migratory birds, though sample preparation methods may affect results. In this study I examined feathers from red-tailed hawks (*Buteo jamaicensis*) to determine the relationship between measure of  $\delta D$  due to inter-feather variation or drying methods, assessed the accuracy of results by using two birds of known-origin and estimated possible natal origins of migratory red-tailed hawks. Two feathers per individual were taken from 81 wild hawks caught at Hitchcock Nature Center near Crescent IA and from 2 rescued red-tailed hawks, Raptor Recovery Nebraska near Eagle, NE. 119 of the collected feathers were used. I found that there was no significant inter-feather variation, and that there was no significant difference between air-drying and oven-drying feathers.  $\delta D$  results of the known-origin birds corresponded with Eagle and the origin of their food (Madison, WI). Maps were constructed using ArcGIS that display possible origins of wild birds and known-origin birds based on  $\delta D$  of their feathers.

## **Introduction**

Biologists study the migratory habits of birds to understand bird life histories. Because birds utilize many different habitats during migration, learning where bird species spend each season gives biologists important insights to what birds need to survive. Staggering numbers of birds and bird species migrate (Gill, 2007), so knowledge of the migratory habits of each species is necessary for the management of game and non-game birds.

There are a number of methods used to study migration, such as mark-recapture (Braun, 2005). Stable isotope analysis of feathers is the newest method of studying bird migration. The first studies that used stable-isotope analysis to monitor bird migration were conducted in 1997

(Chamberlain et. al. 1997, Hobbson and Wassenaar 1997). Isotopes of several elements exist naturally in the world in known abundance ratios (Brenna et al. 1997) and many follow predictable variations. The food and water birds eat and drink contain specific ratios of stable isotopes of elements, which are then deposited in tissues. Bones, fat, muscle and feathers all theoretically have the same isotopic signatures as the food and water birds consumed at the time the tissues were grown (Hobson 1999, 2005).

Stable isotopes of carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ) and deuterium ( $\delta\text{D}$ ) have helped researchers analyze avian nutritional ecology, migratory habits and nutrient allocations in eggs (Hobson 2005, 2006), so the potential for this technology to aid in avian studies of many sorts is great. For migration, deuterium is a favored isotope because hydrogen isotope abundance ratios reflect continental precipitation patterns (Hobson, 1997).  $\delta\text{D}$  values from feathers can be compared to maps showing  $\delta\text{D}$  of precipitation ( $\delta\text{D}_p$ ) to estimate the geographic origin of birds. This comparison is possible due to the nature of hydrogen in precipitation.  $\delta\text{D}_p$  values change with a more or less north south gradient, and most birds migrate north to south. Because birds migrate against the  $\delta\text{D}_p$  gradient, the values are useful in determining where a bird grew its feathers.

Birds that migrate typically molt their feathers before a major migration in the fall or spring. New feathers grown before migration will have a distinct  $\delta\text{D}$  value to the area the feathers were grown in. Also, because feathers are metabolically inactive once fully grown, feathers grown in a single area will have a  $\delta\text{D}$  signature unique to the geographic area they were grown in (Hobson 1999).

The same relationships hold true for other tissues. Blood, muscle and bone tissues all store isotope ratios. The longer it takes a tissue to form relative to bird movements, the less spatially-explicit a signature will be (Yerkes et al 2008). For example, bones take a very long time to form and so a bone sample would likely have  $\delta D$  values that represented deuterium uptake from many locations, and so would be useless to determine migration patterns but may be very useful for determining the overall health of a bird. Muscle and bone form too slowly to use for migration studies, and would require physical harm to birds or the use of hunter-recovered or other dead birds (e.g., roadkills). Blood is formed and replaced quite quickly and can be sampled without harming the subject, but because blood is so metabolically active, any food and water taken in by a bird during migration will show up quickly in the blood and influence the  $\delta D$  value of the sample. Feathers are the favored tissue to determine geographic location because they form relatively quickly and are metabolically inactive once formed.

So why use isotopic analysis? Field studies such as mark-recapture, telemetry and radar were and still are fundamental ways to track migrating birds. However, field studies are “very labour-intensive and expensive” (Powell and Hobson, 2006), and are often dependent on collecting a rather large sample size (Hobson, 2005). Isotope analyses provide a less labor-intensive, cheaper alternative.

Isotope analysis is also advantageous because it allows scientists to learn a great deal about a single bird with little sampling effort. Yerkes et al. (2008) used  $\delta D$ ,  $\delta^{13}C$  and  $\delta^{15}N$  isotopes to compare the overall health of female Northern Pintails (*Anas acuta*) arriving on their breeding grounds to where individuals wintered. Simple blood and feather sampling allowed Yerkes et al. (2008) to correlate body fat content of female pintails to wintering areas and found that females that wintered on freshwater habitats had more body fat upon arrival than those that

wintered in coastal areas. Without the aid of isotopic analysis, this study would have required pintails to be captured in both their wintering and breeding locations, which would have been nearly impossible. Stable isotope analyses are an excellent opportunity to vastly expand the scope and intensity of wildlife management.

Despite its vast potential, stable-isotope analysis is not without its pitfalls. Many assumptions must be made for analyses to be useful, and there have been problems, particularly in raptors, with the reproducibility and comparability of results. Exercise and increased respiration rates may enrich  $\delta D$  in birds (Hobson 2006). Deuterium ratios, across years, may also be influenced by diet (Powell and Hobson 2006). Powell and Hobson (2006) discussed the possibility that differences in food webs resulted in different  $\delta D$  values for individual wood thrushes in two different years. This is especially important in raptors, because as predators there is a greater opportunity for altered deuterium enrichment due to food web changes.

Another problem is using stable hydrogen ratios from precipitation ( $\delta D_p$ ). While using  $\delta D_p$  values enables us to get a clear idea of geographic origin across a continent, it also creates a problem with comparability of results. Maps that summarize  $\delta D_p$  patterns are made from long term means of precipitation. Because any given year or any given area may have different  $\delta D_p$  than the average, the values in animal tissues in reality may be slightly offset from any geographic assignments based on these maps (Bowen, 2009).

The first goal of this research is to assess the reproducibility of stable hydrogen isotope analysis when studying raptors. Continuing to use isotope analysis without addressing the issues facing the methodology will result in unreliable data being produced. However, in order to

conquer the problems facing this technology much research needs to be done to pinpoint the exact problems and derive solutions to them. I hope to aid in this process through this project.

By looking at the results of stable isotope analysis on birds of known origin, a number of things can be learned. First, results will allow us to determine how precise and accurate analyses of feathers are. If there is error present in deuterium analysis, a pattern of error may be present. By analyzing results and comparing them to correct geographic origin, we can determine if any errors in analyses are random or not, which will improve accuracy in analyzing results for birds of unknown origin. Duplicate samples from the same individual will help assess the reproducibility of isotopic analyses within a single lab. If deuterium analysis is consistent, both feathers from any bird should yield the same  $\delta D_f$ . A study of the reproducibility of results between multiple labs is beyond the scope of this project.

The second goal is to determine if sample preparation methods of feathers change  $\delta D_f$ . Paritte and Kelly (2009) found that cleaning methods consistently affected  $\delta D_f$  of quail feathers. To further assess ways that preparation can alter  $\delta D_f$ , I will compare air-drying and oven-drying feathers after cleaning.

The third goal is to assign geographic origin to migrating *Buteo jamaicensis* (red-tailed hawks). There are few states in the Great Plains and Midwest that monitor raptor migration. States that have Hawk Watch sites include Colorado, Iowa, Michigan, Minnesota, Texas and Wisconsin (Hawk Migration Association of North America, 2010). There are few states that monitor migration in the mid latitudes of the Great Plains and Midwest, making the sites in Colorado and Iowa more important. By learning more about where hawks that migrate through

Iowa (where samples will be collected), I can better understand how raptors move from northern states to southern states.

## **Literature Review**

The goal of this study is to assess whether stable hydrogen-isotope analysis can help estimate geographic origin of migratory birds, especially raptors. A recent paper by Smith et al. (2009) found serious problems with reproducibility of results with raptor feathers. Smith et al. (2009) felt so strongly about the negativity of their findings on the field that “we caution against the continued use of  $\delta D_f$  for predicting geographic origin, and for addressing important conservation questions”.

This research is important because isotope analysis is important to wildlife management. Powell and Hobson (2006) used isotopes to track dispersal of Wood Thrushes, Delong et al. (2005) used isotopes to track movements of Flammulated owls, a species whose movements were previously unknown. Yerkes et al. (2008) used stable isotopes to track origin and health of migrating Alaskan Northern Pintails and were able to derive relationships between health upon arrival at breeding sites and habitat type used before migration.

Stable isotope analysis has many applications in wildlife, but it is still a relatively new method of study, with the first applications in migration in 1997 (Chamberlain et. al. 1997, Hobbson and Wassenaar 1997). As with all technological advancements, stable isotope analysis has some problems. I agree with Smith et al. (2009) that there appear to be problems that need to be addressed, but I disagree with their caution against further studies. Techniques cannot improve unless they are used, and the purpose of my own research is to add to the growing amount of literature and research involving stable isotopes.



An example in a closely related field can be found in Brenna et al. (1997) who discussed how, in the early 1990s, mass spectrometry took a step forward. Mass spectrometry remained “remarkably unchanged for four decades”, until the introduction of high precision continuous-flow isotope ratio was introduced in the 1990s. Mass spectrometry had been used for a number of purposes in its early years, but with the introduction of high precision continuous-flow technologies, the applications of mass spectrometry increased dramatically. Stable isotope analyses may see similar advances in the near future.

I chose to study raptors because there seems to be the most uncertainty in reproducibility of results with raptor feathers compared to other birds. Several authors have discussed the possibility that  $\delta D$  may change with trophic levels (Meehan et al. 2003, Hobson 2005, Powell and Hobson 2006). These authors suspect that  $\delta D$  values present in feathers may be influenced by diet. Raptor feathers may be especially vulnerable to compromised  $\delta D$  values because they are secondary consumers. The red-tailed hawk is a very common raptor, and it is found almost everywhere in the United States and much of Canada (Wheeler, 2003). I chose a common bird to ensure an adequate sample size. Though the red-tailed hawks are common, the results of this study can be applied to other raptors.

Raptors such as the Bald Eagle (*Haliaeetus leucocephalus*) are species of national concern. Some birds of prey, such as the California Condor (*Gymnogyps californianus*) are listed as endangered or threatened (IUCN, 2010). Studying these birds is important to ensuring their survival, and if stable isotope analysis can aid scientists in their research it should be pursued.

Studying raptors during migration is the easiest way to study them. During winter and summer months, most raptors have low densities in any given area. During migration, their densities are very high so studying them is much easier and more reliable due to larger sample sizes.

Some of the first wildlife applications of stable isotope analysis were published by Hobson and Wassenaar (1997), and Chamberlain et al (1997). Both studies investigated using hydrogen (and others by Chamberlain et al.) to track migrating birds. After these studies were published, many others began using stable isotopes, especially hydrogen, to track the migration patterns of birds.

It is clear that using stable-hydrogen isotopes has great potential, but also that it holds uncertainties that make the comparison of data across different studies inadvisable until standard preparation methods can be developed. The reasons for this include: lack of consistent standards among laboratories, the use of the Global Network of Isotopes in Precipitation (GNIP) data sets, and the trophic level effects discussed previously.

The GNIP is a data set produced by the International Atomic Energy Association that characterizes regional patterns of deuterium concentrations in precipitation. While extremely helpful, caution must be used when comparing results to the GNIP data because the data is a long-term average. Precipitation obviously changes yearly from place to place, and so even if a  $\delta D$  value is correct for a given feather, the location assigned may be skewed due to seasonal or yearly variations in precipitation. This makes reliably comparing data from different regions and different years difficult (Hobson, 2005). Also, GNIP data is limited by the number of observation stations that measure  $\delta D_p$ . While there are hundreds of sites observed globally, the

world is a big place and there are some areas in the world that are less accurate than others (Bowen 2009).

A lack of standardized laboratory methods is an issue. It was this fact that spurred Smith et al. (2009) to warn against using stable hydrogen isotope analysis at all until “the factors of poor reproducibility are identified and improved reproducibility is demonstrated within and among laboratories”. Smith et al. (2009) found substantial differences in results within and between two separate laboratories. I hope to address the issue of discrepancy of results. Smith et al. (2009) ran multiple repeats of samples in two different labs. While the size and time Smith et al. (2009) took to collect their data is beyond the means of my own research, I can replicate this idea on a smaller scale. I plan to collect a feather from each sample bird and run duplicates of the feather. My results will evaluate the recommendations of Smith et al. for intra-laboratory consistency problems. Conducting a larger study similar to Smith et al. (2009) using multiple laboratories may be feasible for graduate research in the future.

## **Methods**

*Sample collection:* Feathers from 89 red-tailed hawks were collected for this study, and two feathers were collected from each bird, and 130 feathers were analyzed. Two birds were local residents, and 87 were migrants. Migrant feathers were collected from migrating hatch-year (juvenile) hawks at the Hitchcock Nature Center near Crescent, Iowa from August through October 2010.

We collected two body feathers from the upper right leg from each bird promptly after capture. We placed leg bands on each bird, and then placed feathers in sealed envelopes marked with date of capture and the band number. We stored feathers in the bird blind through October 31, then brought them to the laboratory where they were then stored in desiccators to prevent ambient moisture contamination. Body feathers were used instead of flight feathers to eliminate any potential of negative impact on

migration. As only juvenile birds were analyzed (with the exception of known-origin hawks), I assumed that body feathers were grown at the same time as flight feathers. Resident feathers were collected from two hawks at Raptor Recovery Nebraska (RRN), which is located approximately 1 mile west of Eagle, NE, or about 5 miles east of Lincoln, NE. The two hawks from RRN are adults, but have resided at RRN since 2003 and 2005.

*Lab analyses:* Isotopic analysis was conducted at the University of Nebraska, Lincoln Water Science Laboratory. I washed feathers in a 1:30 solution of Fisher Versa-Clean detergent: distilled, deionized water (DDI water) and rinsed them three times in three 600 mL beakers of DDI water, following the methods outlined by Paritte and Kelly (2009). The beakers of DDI water were replaced after 5 feathers were rinsed. Feathers were dried according to the methods of experiment one or three (see Figure 1). For the second wash, I used a 2:1 chloroform:methanol solution to remove lipids and dried them in the fume hood or oven. The clean feathers were clipped at the very tip with scissors and 0.20mg of the feather was weighed into silver (Ag) foil cups.

We conducted hydrogen pyrolysis using a glass carbon high-temperature (1260° C) elemental analyzer (Eurovector EA 3000, Eurovector Corporation, Milan Italy), interfaced to an Isoprime stable-isotope mass spectrometer. Pyrolyzed feathers were analyzed for hydrogen content and isotope abundance with an electrostatic analyzer.  $\delta D$  values obtained by analysis are reported in units of per thousand (‰) and normalized using the Vienna Standard Mean Ocean Water—Standard Light Antarctic Precipitation (VSMOW-SLAP). We used organic keratin reference standards of known deuterium content purchased from Leonard Wassenaar, at the Stable Isotope Hydrology and Ecology Research Laboratory, University of Saskatchewan in Saskatoon., Canada. Raw  $\delta D$  results from samples were corrected using these standards.

The goal of experiment one was to assign geographic origin to the birds sampled. The goal of experiment two was to determine if there was significant intra-feather variation of  $\delta D$  in individual

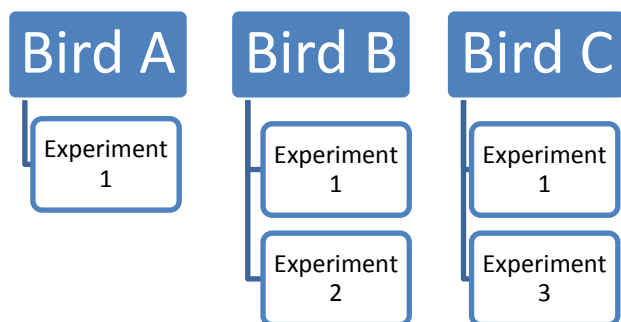
hawks. The goal of experiment three was to determine if there is significant difference between drying methods on  $\delta D$ . Drying methods for each experiment are discussed below.

Experiments 1 and 2: I dried feathers in an oven at 65 degrees Celsius for 2 hours after each wash cycle. Feathers were stored in desiccators when they were not being dried or cleaned.

Experiment 3: I dried feathers in a fume hood at room temperature for 24 hours after each wash cycle. Feathers were stored in desiccators when they were not being dried or cleaned. Linear regression analysis was conducted for experiment three to determine if enrichment effects of drying methods were present (see Figure 4).

Each feather was assigned to one of three groups: the primary sample group, duplicates or air-dried duplicates. Intra-feather variation was assessed by using experiments one and two, while drying methodology was assessed using experiments one and three. Primary samples were used in all three groups. An individual hawk could donate feathers to only experiment one, or to experiment one and a second experiment. Figure 1 shows possible combinations of experiments a single bird could donate feathers to.

**Figure 1** Example of three potential sample distributions among experiments of stable isotope analyses conducted on red-tailed hawks from Iowa and Nebraska during 2011. Each non-filled box represents a feather from an individual bird.



*Geographical Analysis:* Three maps showing deuterium gradients across North America were made using ArcGIS. Maps were constructed using base maps of monthly precipitation values from May to September. Red-tailed hawks can begin nesting as early as January in southerly locations (south of Colorado) or as late as July in Alaska and northern Canada (Wheeler 2003). Wild hawks were assigned a range of likely origin based on lab analysis of feathers and typical range of the individual's subspecies. Borealis (*B.j. borealis*), Borealis pale-morph (Krider's) and Calarus (*B.j. calarus*) subspecies of red-tailed hawk nesting typically ends June through August, but birds in lower altitudes or more southerly locations may conclude nesting earlier (Wheeler, 2003). Harlan's hawks (*B.j. harlani*) conclude their nesting in August and September (Wheeler, 2003).

I constructed monthly maps for the months of May through September to correspond with the end of the nesting season (see Appendix B), and  $\delta D_f$  values for individuals were compared to maps that corresponded with months the individual likely left the nest based on subspecies. For example, I compared the  $\delta D_f$  of the Harlan's hawk to maps from August and September because it is unlikely that the hawk fledged before August. To map the RRN birds, I made a map using monthly deuterium averages. I chose to use annual monthly averages because the control birds are adults, and there is limited information about when and how often adults molt contour feathers (Poole et al 1993).

I also used political boundaries and land cover layers in the GIS analysis for visual reference. These layers were obtained from the Commission for Environmental Cooperation (2005).

*Statistical Analysis:* F-tests were conducted to determine if the samples in each experiment were normally distributed. Samples were not normally distributed, so I used a Behrens-Fisher test ( $t'$  test) was used to determine if 1) Samples in experiment two were significantly different and ( $H_0: \mu_1 = \mu_2$ ) 2) Samples in experiment three were significantly different ( $H_0: \mu_1 = \mu_2$ ). I used a confidence level of 95% ( $\alpha = 0.05$ ) for all statistical tests. To further analyze the comparability of drying methods, I conducted a linear regression comparing the methods. I also

conducted a linear regression to compare  $\delta D$  and capture date. We hypothesized that birds from more northern latitudes would migrate later in the year, and would be caught later in the season.

## **Results**

Estimated  $\delta D$  values for each individual are listed in Appendix A. Constructed maps are shown in Appendix 2. Tables 1-3 display the mean, variance, standard deviation, coefficient of variation and standard error of the mean for each experimental group.

**Table 1: Primary Samples (Experiment 1)**

	Primary Samples (Oven-dried)
Mean	-66.69
Variance	646.6413774
Standard Deviation	25.42914425
Coefficient of variation	0.381281033
Standard Error of Mean	2.843064758

**Table 2: Duplicate and Primary Samples (Experiment 2)**

	Duplicate Samples (Oven-dried)	Primary Samples (Oven-dried)
Mean	-86.40	-86.51
Variance	1047.544834	945.9667
Standard Deviation	32.36579728	30.75657

Coefficient of variation	0.374603372	0.35552
Standard Error of Mean	13.21328141	12.55632

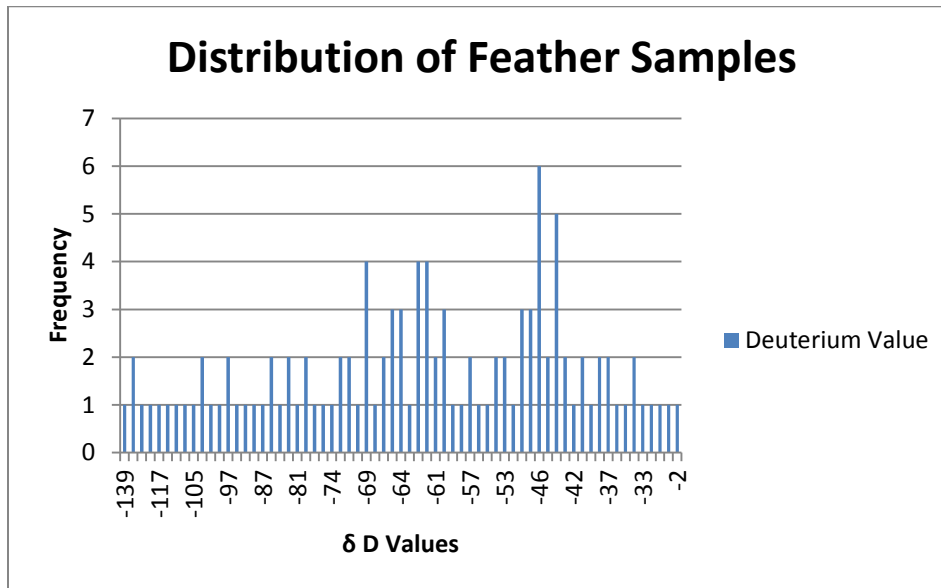
**Table 3: Air-dried and Oven-dried Samples (Experiment 3)**

	Air-dried Samples	Oven-dried (Primary) Samples
Mean	-53.84	-60.68
Variance	229.07	312.14
Standard Deviation	15.14	17.67
Coefficient of variation	0.28120357	0.291199736
Standard Error of Mean	3.473354211	4.053776017

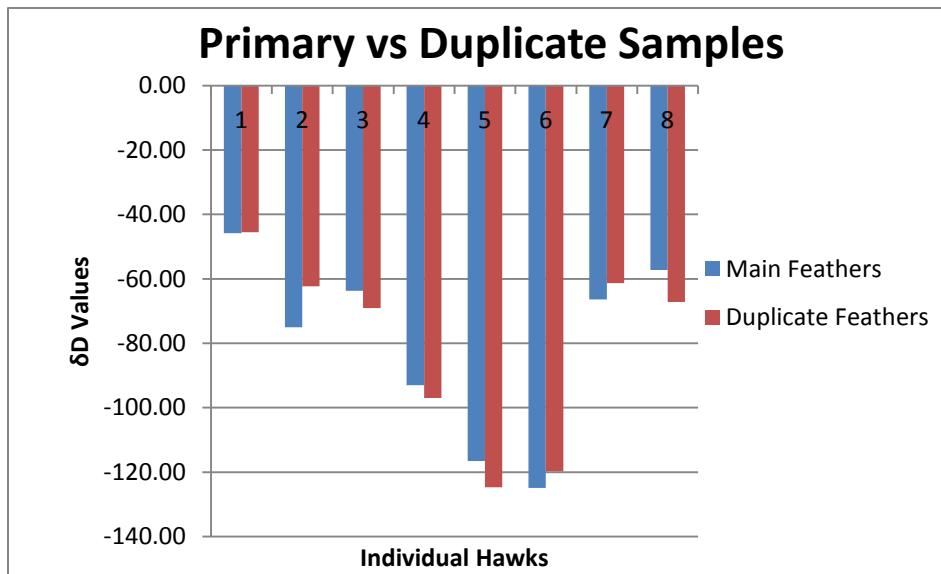
I used  $\alpha=0.05$  for t' tests. Experiment two compared primary samples and duplicate samples prepared with the same drying methods. The critical t value for experiment two was 2.02, and  $t = 1.55$ , so the null hypothesis was not rejected (duplicate samples and primary samples do not differ significantly). Experiment three compared oven-dried primary samples and air-dried duplicate samples. For the third experiment, critical  $t = 1.69$  and  $t = 1.25$ . Because the absolute value of t was less than critical t, the null hypothesis was not rejected (drying methods are not significantly different).



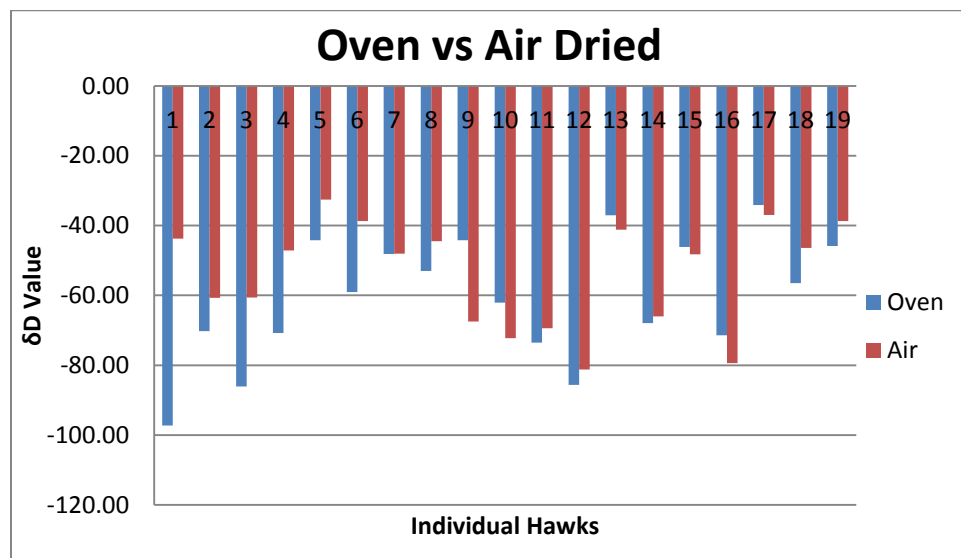
**Figure 2 Distribution of all samples**



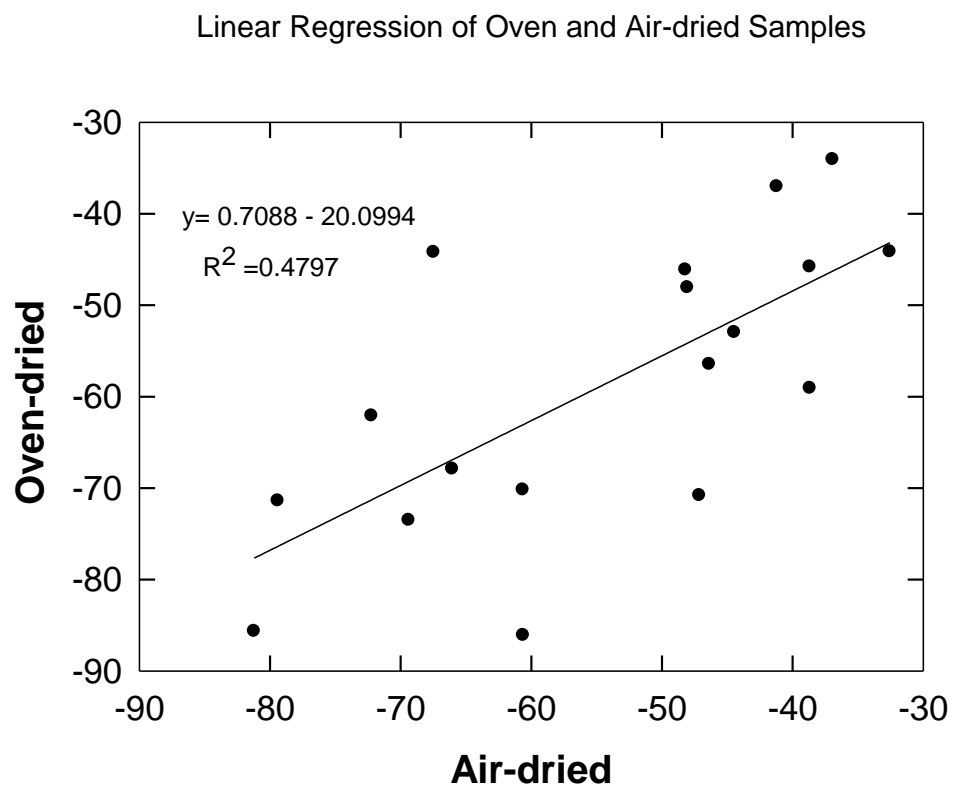
**Figure 3 Primary vs Duplicate Feather Samples**



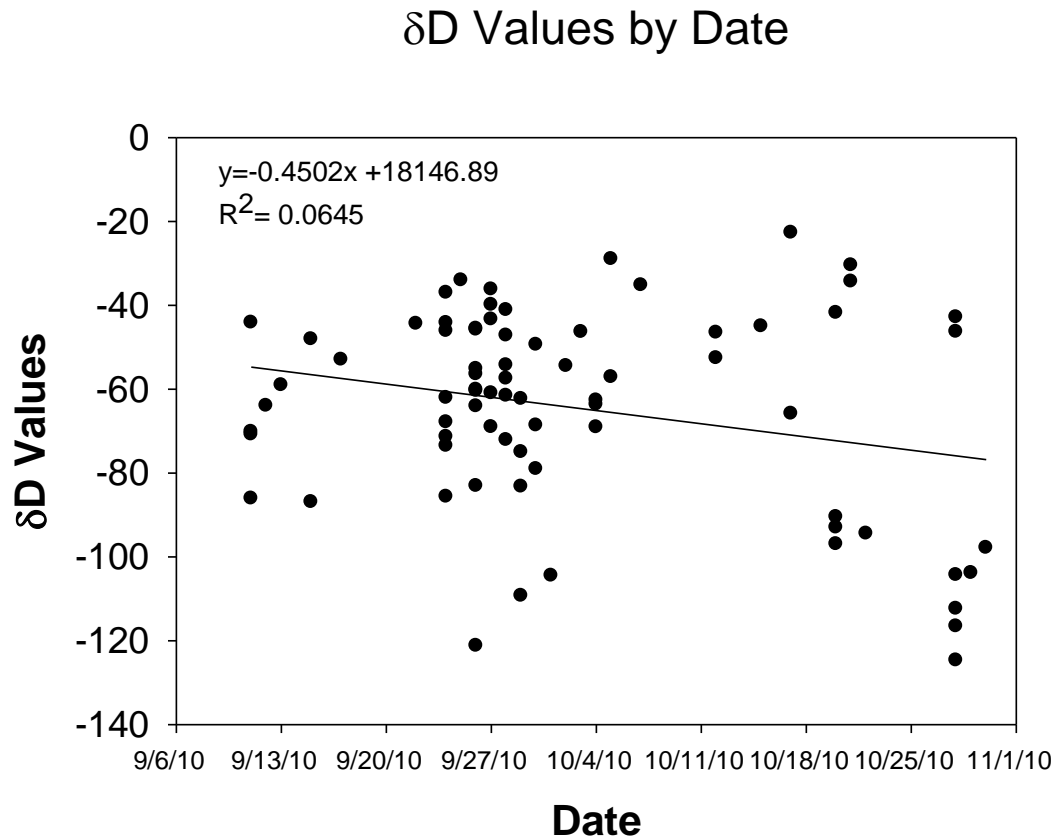
**Figure 4 Oven-dried feathers compared to air-dried feathers**



**Figure 5 Linear Regression Analysis of Oven-dried and Air-dried samples**



**Figure 6:  $\delta D$  by Capture Date**



The maps in Appendix 2 show birds of special interest. The vast majority of red-tailed hawks that are caught and banded at Hitchcock are the Borealis subspecies. I created maps with polygons of likely nesting area for hawks that are not commonly caught at Hitchcock. These birds included the Calarus and Harlan's subspecies, Krider's hawks, and Borealis hawks that likely nested south of Hitchcock.

Two Krider's hawks were captured, and they had mixed results. One (band number 1957-00707) had a  $\delta D$  value that placed it well within its normal summer range in Montana. The second (band number 1957-00772) had a  $\delta D$  value that placed the hawk in its winter range. One Calarus subspecies (band number 1967-05778), had a  $\delta D$  value of -55, which placed the hawk in

California. While this is well within the normal range of Calarus hawks, it seems unlikely that a bird migrated so far east and north to reach Hitchcock. It is possible that these feathers were not completely dried (removing all cleaning solutions) or that I made other errors in sample preparation.

## **Discussion**

The results of this study are encouraging. A lack of inter-feather variation demonstrates that any contour feather on a juvenile bird will give a representative estimate of  $\delta D$ . Using Paritte and Kelly's (2009) methods of sample preparation, 48 hours are needed to dry each sample (two 24 hour cycles). Because drying methods did not vary significantly, preparation time of samples can be reduced from 48 hours of air-drying to 4 hours of oven-drying.

Though drying methods were not found to be significantly different, it should be noted that I observed a low  $R^2$  value (0.4797) in the linear regression analysis. A slope of 1 would indicate that the two drying methods were completely comparable, and the resulting slope from the regression analysis was 0.7088, close to 1. However, with an  $R^2$  value of less 0.5, less than 50% of the points are determined by this slope (Zar, 2010). The chance of a type one error is small but not impossible. I recommend future studies analyze be conducted to re-analyze differences in drying methods.

However, as previously mentioned, this study has not addressed the reproducibility between laboratories discussed by Smith et al (2009). I feel that more studies need to be conducted to standardize laboratory preparation and analysis methods to fully address these concerns.

RRN birds are fed with a combination of local food and food from Rodent Pro (Gainesville IN). RRN raises some rodents and feeds all rodents food from Madison WI. Meehan et al. (2003) discussed how adult and juvenile hawks can have different deuterium ratios, even when they have the same food source. While this is potentially a problem, adult birds were chosen for two reasons. RRN treats wild birds that are injured and provides permanent care for birds that cannot be released back into the wild. However, both birds, named Rusty and Shadowfax, have been with RRN since 2005 and 2003, respectively. Red-tailed hawks may not completely molt feathers (Poole et. Al. 1993) every year as adults, but I assumed that since both birds have been at RRN for at least 5 years they have completely molted their feathers at least once at RRN.

Rusty and Shadowfax had an average  $\delta D_f$  of -62‰ and -63‰ respectively. Map 8 shows that both birds are from areas just north of Madison or just northwest of Lincoln. Because Lincoln and Madison fall in the same range of  $\delta D$ , it is difficult to determine by deuterium content which of the two locations the birds are likely from. Raptor Recovery Nebraska feeds hawks with a mixture of rodents from Gainesville, IN, and rodents raised at Eagle with food from Madison.

Maps show Lincoln rather than Eagle for two reasons. First, Eagle is 14 miles due east of Lincoln and it is unlikely that there is much difference in  $\delta D$ . Second, Lincoln is a much more recognizable landmark than Eagle. Given the range of error, the  $\delta D_f$  of both birds provides an accurate of their molt location.

## **Summary and Conclusions**

This study was conducted to contribute a broader understanding of how stable-isotopes can be used to study raptors. My findings should encourage further exploration of sample preparation methods and more positive attitudes about the future of this field than those of Smith et al (2009).

If I could do this project over again, I would run more duplicate samples. To do this, I would collect three feathers from each bird, and then prepare two with one drying method and the third with a second drying method. I would also do a more comprehensive study on birds of known-origin. I would sample the water and food they consume as well as the air in addition to the feathers. This would allow me to isolate how hydrogen isotopes fractionate as they travel through a food web and would give additional data on the accuracy of using  $\delta D_f$  to estimate origin. It would also allow for larger sample sizes to be examined, making statistical analyses more robust.

Future studies might also analyze both feathers and blood for  $\delta D$ . Blood is very metabolically active and would grant us yet another possible data point. With the  $\delta D$  estimate from feathers, blood and the origin of capture, we might be able to get an even better picture of an individual's migration route.

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## Appendix A

Table 4 Primary Samples (Oven-Dried)

<b>Band Number</b>	<b><math>\delta D</math></b>	<b>Band Number</b>	<b><math>\delta D</math></b>
1957-00759	-97.27	1967-05772	-52.98
1957-00760	-70.20	1967-05773	-44.43
1957-00761	-86.11	1957-00768	-44.22
1967-05769	-70.81	1957-00769	-62.09
1967-05770	-44.15	1957-00770	-73.53
1957-00763	-63.99	1957-00771	-85.66
1957-00764	-59.08	1957-00772	-37.04
1957-00765	-86.94	1967-05774	-67.91
1957-00767	-48.09	1967-05775	-46.14
1957-00778	-83.11	1957-00783	-57.51
1967-05778	-55.18	1957-00784	-47.22
1957-00779	-39.91	1957-00785	-72.14
1967-05779	-36.22	1957-00786	-54.31
1957-00780	-43.38	1957-00788	-75.03
1687-33636	-60.98	1957-00789	-83.28
1967-05780	-69.06	1687-33637	-109.32
1957-00782	-61.57	1957-00790	-49.42
1967-00781	-41.14	1967-05783	-79.08
1177-20865	-34.35	1957-06582	-59.58
1687-33643	-94.46	1957-06591	-61.69
1687-33644	-103.86	1957-00711	-78.47
1177-20872	-42.85	1957-00702	-44.83
1177-20873	-104.35	1957-00707	-84.94
1177-20871	-46.33	1687-33614	-98.61
1177-20870	-116.56	1687-33621	-124.92
1177-20869	-112.37	1687-33626	-108.19
1177-20876	-97.88	1687-33631	-138.86
1957-00773	-71.40	1967-05778	-55.18
1967-05776	-34.07	1957-00779	-39.91
1687-33634	-56.46	1967-05776	-34.07
1957-00774	-45.82	1687-33634	-56.46
1957-00775	-60.43	1957-00774	-45.82
1957-00776	-64.11	1957-00775	-60.43
1687-33635	-121.23	1957-00776	-64.11
1957-00777	-60.14	1687-33635	-121.23
1957-00778	-83.11	1957-00777	-60.14
1957-00792	-68.64	1177-20855	-52.64

1957-00793	-104.50	1177-20856	-46.52
1957-00794	-54.50	1177-20859	-45.00
1957-00796	-46.38	1687-33641	-65.85
1957-00797	-62.71	1177-20861	-22.70
1957-00798	-63.69	1177-20862	-93.04
1957-00799	-28.98	1177-20863	-41.83
1957-00800	-57.15	1687-33642	-90.48
1177-20853	-35.21	1967-05788	-30.47
1177-20865	-34.35	1957-06582	-59.58
1687-33643	-94.46	1957-06591	-61.69
1687-33644	-103.86	1957-00711	-78.47
1177-20872	-42.85	1957-00702	-44.83
1177-20873	-104.35	1957-00707	-84.94
1177-20871	-46.33	1687-33614	-98.61
1177-20870	-116.56	1687-33621	-124.92
1177-20869	-112.37	1687-33626	-108.19
1177-20876	-97.88	1687-33631	-138.86

Table 5 Air-Dried Samples and Oven-Dried Primary Samples

<b>Band Number</b>	<b>Air-dried <math>\delta D</math></b>	<b>Oven-dried <math>\delta D</math></b>
1957-00759	-43.76	-97.27
1957-00760	-60.64	-70.20
1957-00761	-60.62	-86.11
1967-05769	-47.13	-70.81
1967-05770	-32.55	-44.15
1957-00764	-38.67	-59.08
1957-00767	-48.04	-48.09
1967-05772	-44.45	-52.98
1957-00768	-67.46	-44.22
1957-00769	-72.22	-62.09
1957-00770	-69.38	-73.53
1957-00771	-81.21	-85.66
1957-00772	-41.20	-37.04
1967-05774	-66.04	-67.91
1967-05775	-48.19	-46.14
1957-00773	-79.39	-71.40
1967-05776	-36.93	-34.07
1687-33634	-46.38	-56.46
1957-00774	-38.68	-45.82

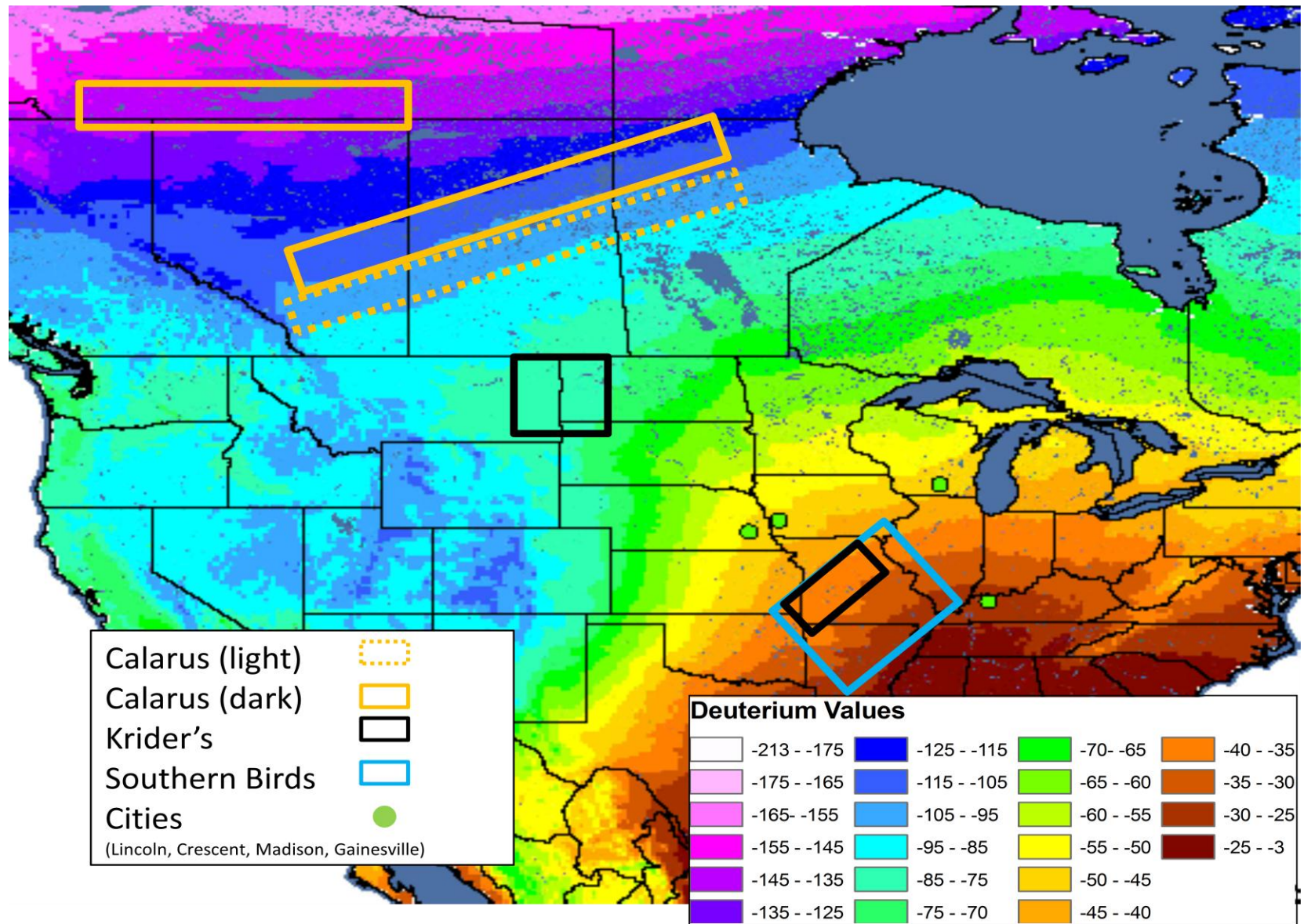
Table 6 Primary Samples and Duplicate Samples (Oven-Dried)

<b>Duplicate samples</b>	<b>Primary <math>\delta D</math></b>	<b>Duplicate <math>\delta D</math></b>
1957-00774	-45.82	-45.56
1957-00788	-75.03	-62.33
1957-00798	-63.69	-69.09
1177-20863	-93.04	-96.98
1177-20869	-116.56	-124.71
1687-33626	-124.92	-119.74

Table 7 Raptor Recovery Nebraska Samples (Oven-Dried)

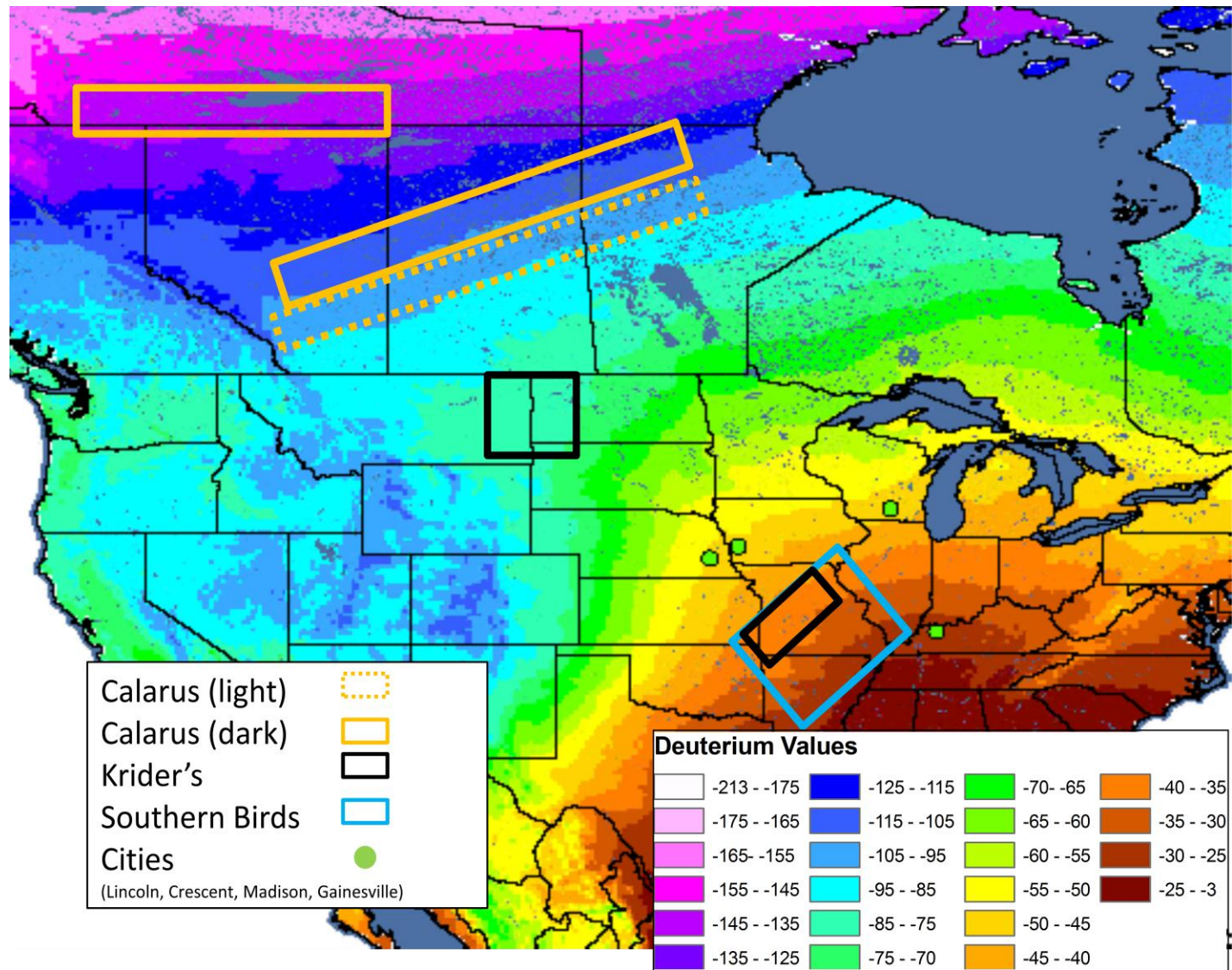
<b>Bird</b>	<b>Primary <math>\delta D</math></b>	<b>Duplicate <math>\delta D</math></b>
“Rusty” (Borealis morph)	-66.43	-66.43
“Shadowfax” (Krider’s morph)	-57.24	-57.24

Map 1: May

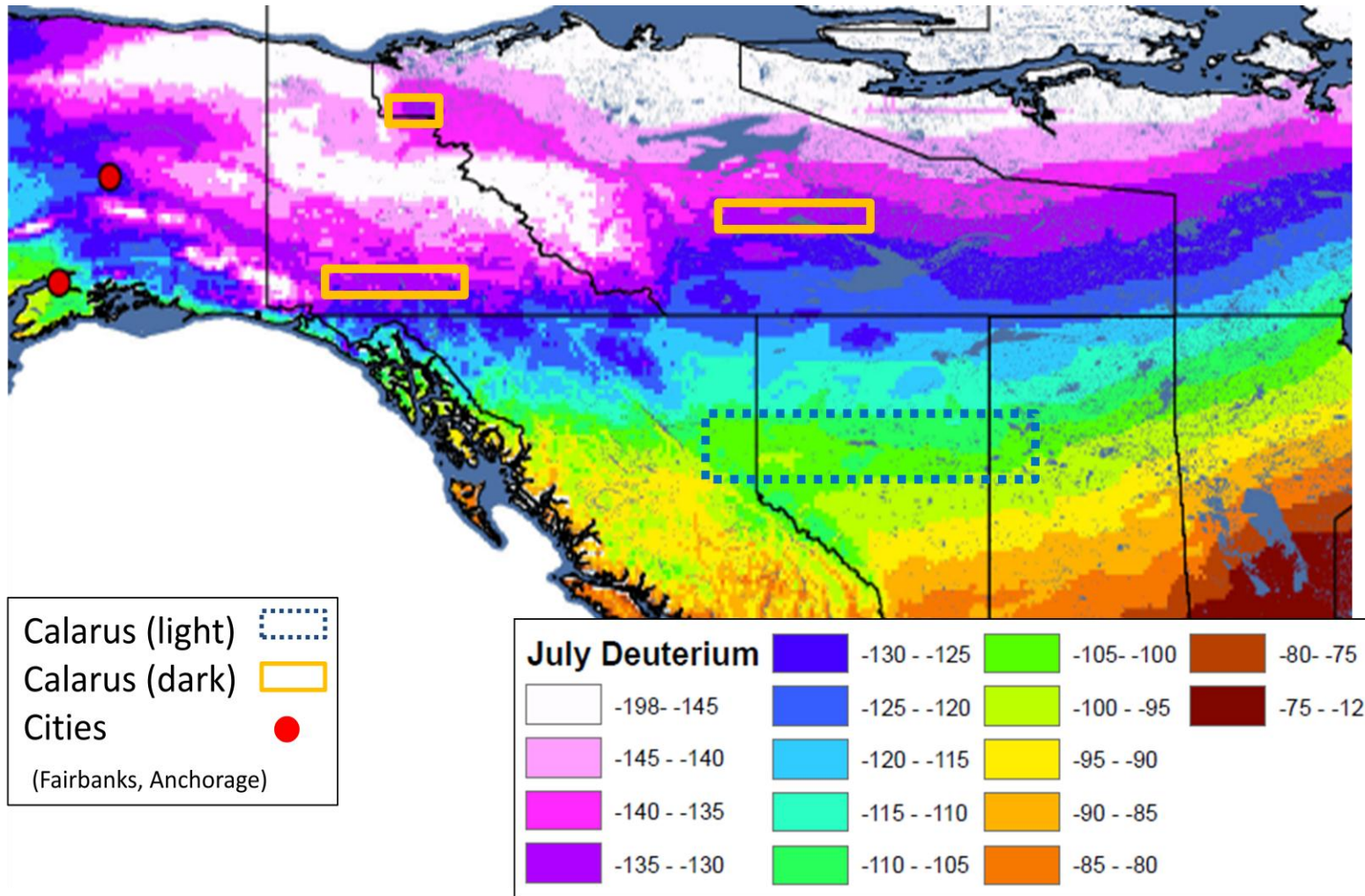




Map 2: June

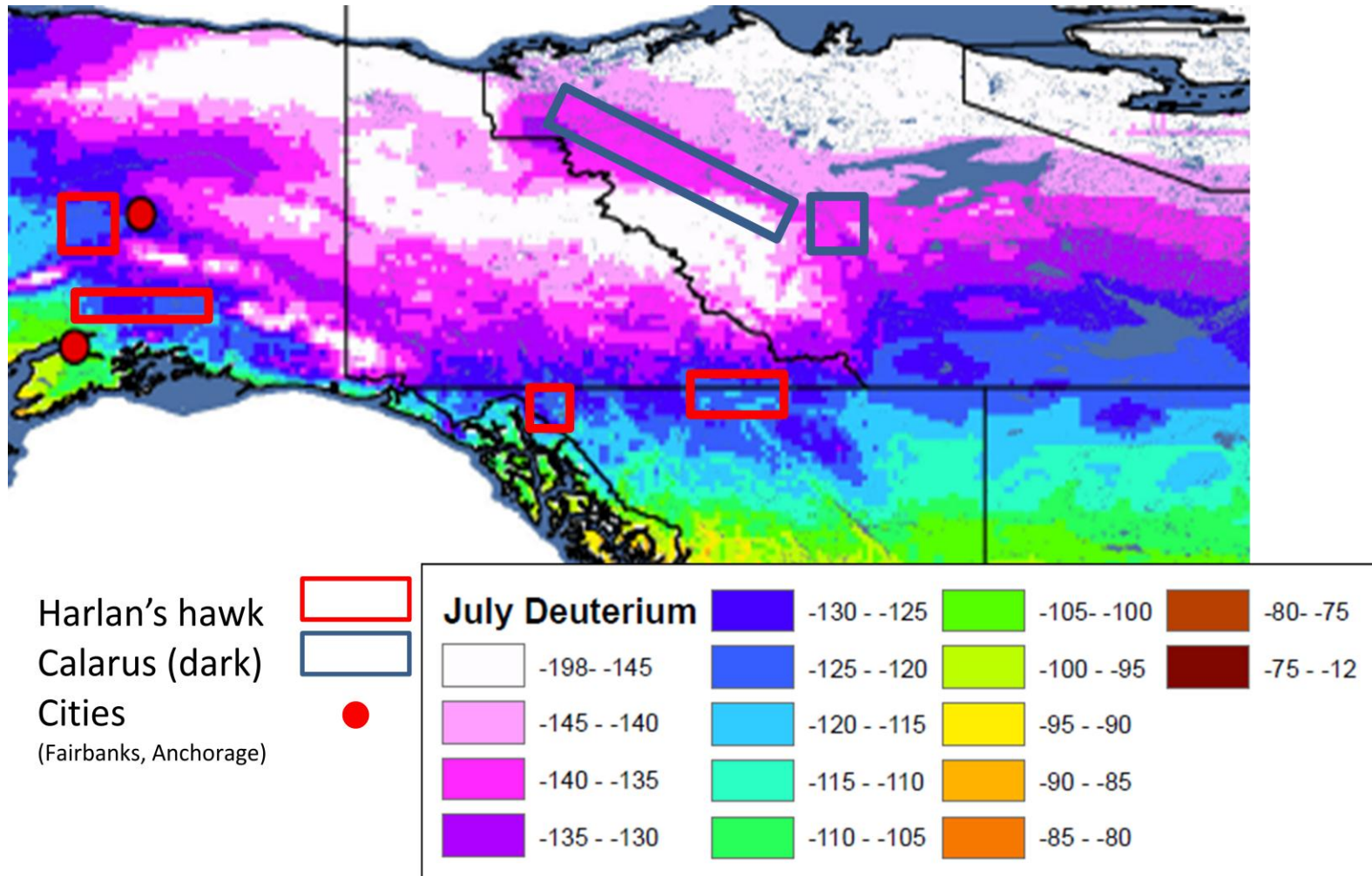


Map 3: July

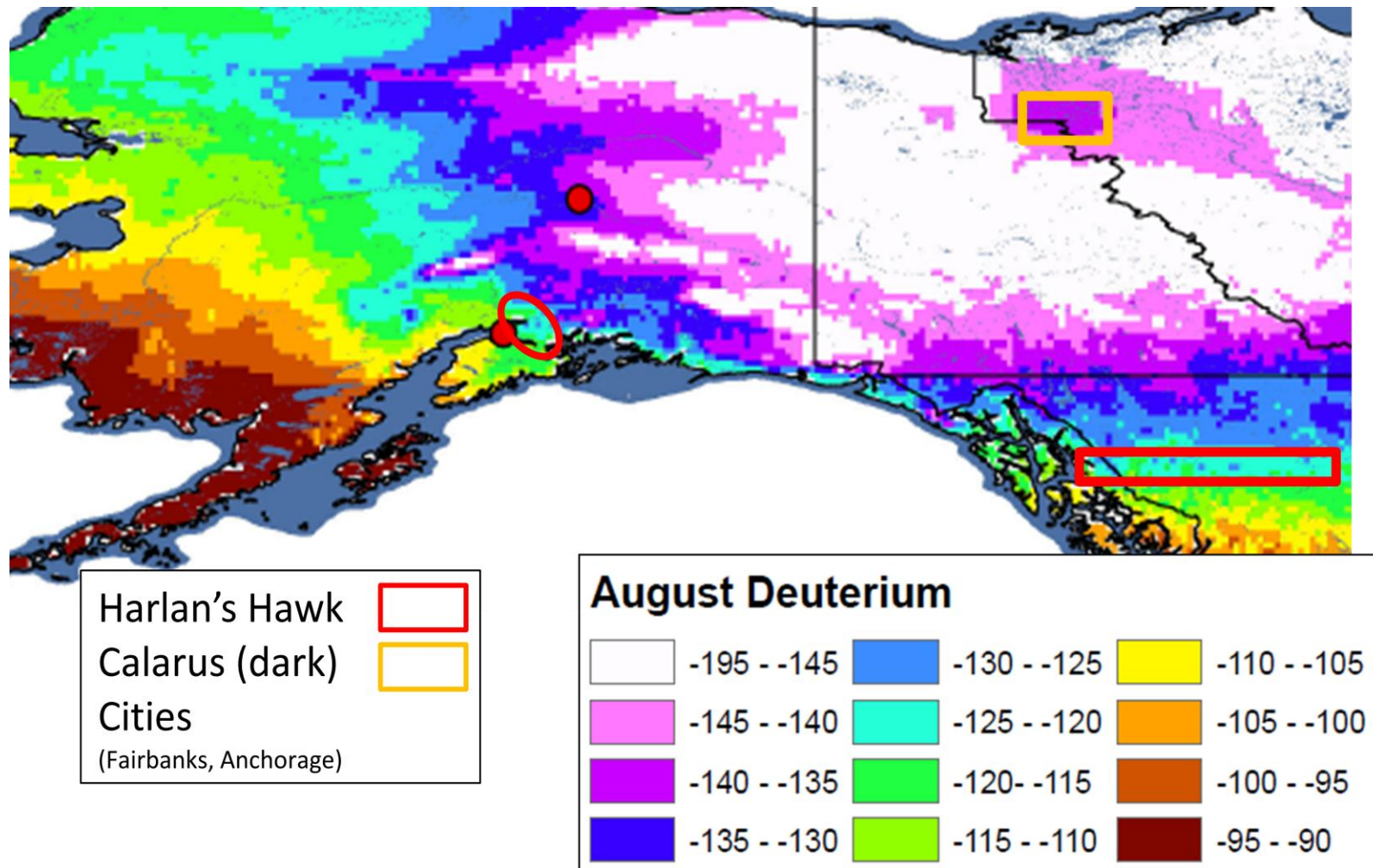




Map 4: July

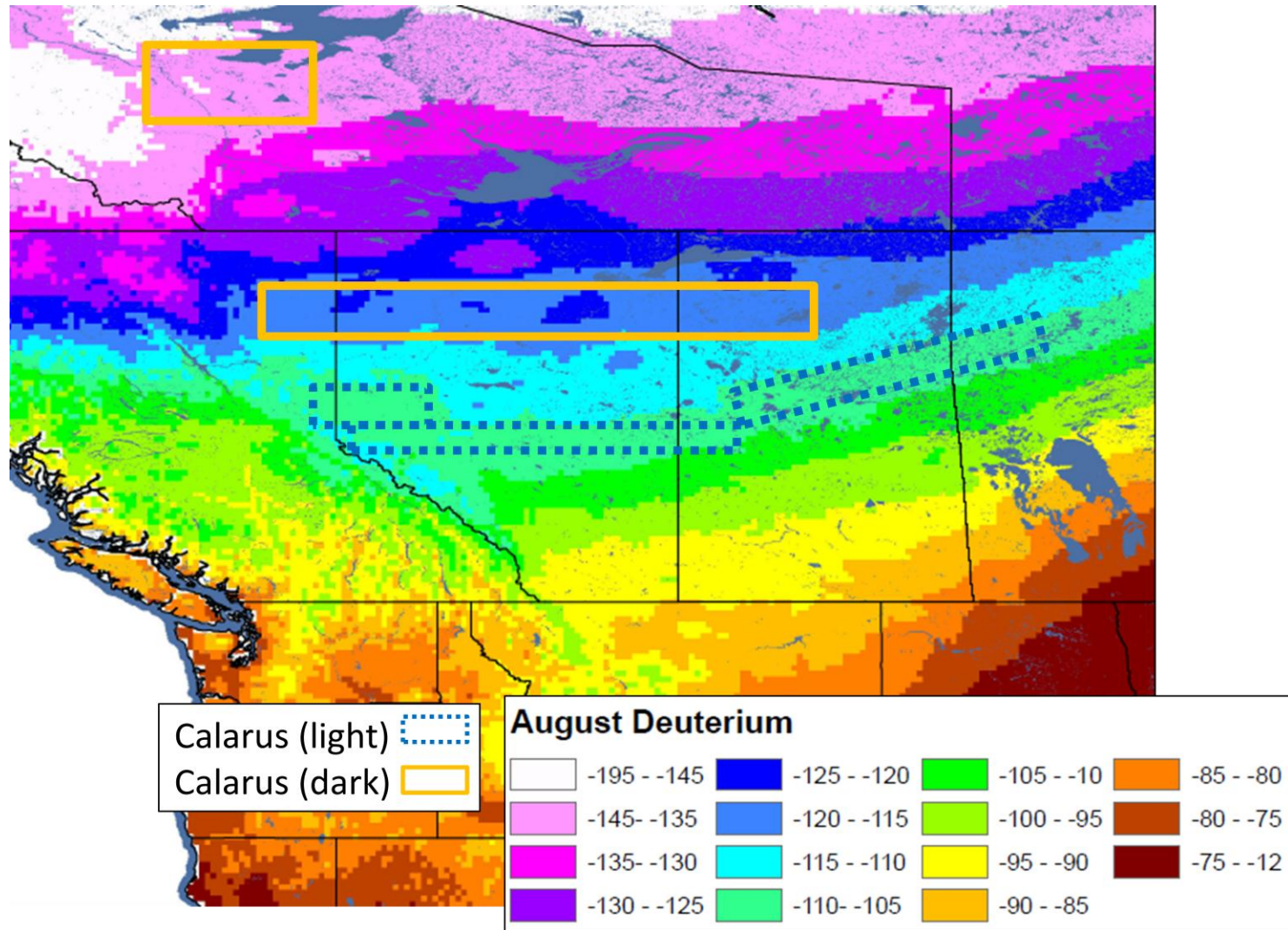


Map 5: August

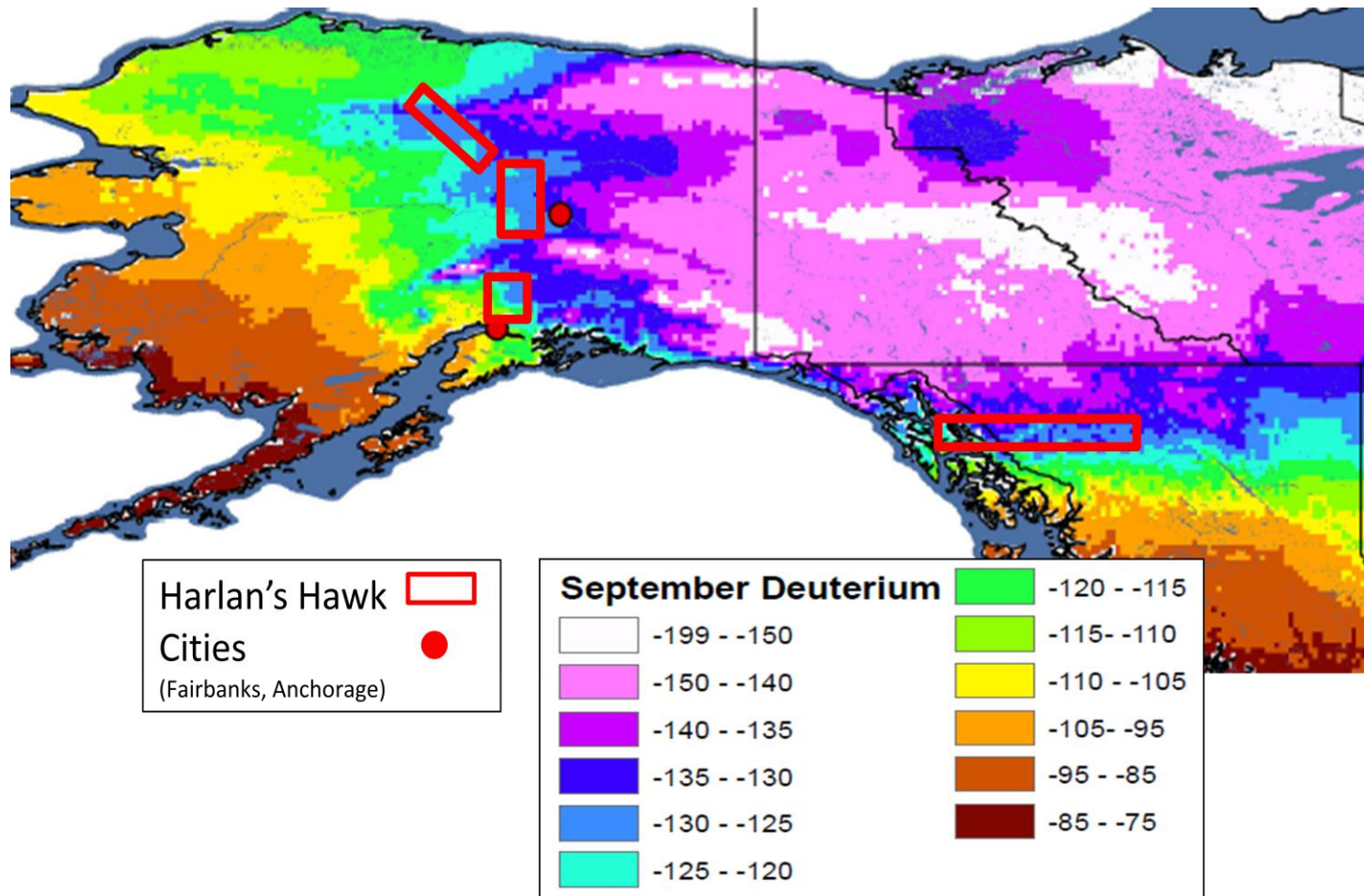




Map 6: August



Map 7: September



Map 8: Monthly Average

